

## CLAIMS OF THE INVENTION

That which is claimed is:

- 5 1. A recombinant double stranded RNA phage (rdsRP) encoding a double stranded RNA eukaryotic expression cassette for expression in eukaryotic cells, the rdsRP comprising:  
at least one segment of a double stranded RNA phage (dsRP) and an internal ribosome entry site (IRES) nucleotide sequence incorporated into the at least one segment of the dsRP.
- 10 2. The rdsRP according to claim 1, further comprising at least one passenger gene sequence incorporated into the at least one segment of the dsRP.
3. The rdsRP according to claim 1, wherein the IRES is inserted into at least one of three dsRNA segments of the dsRP.
- 15 4. The rdsRP according to claim 2, wherein the passenger gene and the IRES are functionally linked.
5. The rdsRP according to claim 3, wherein the segment of dsRP include segment L, segment M or segment S.
- 20 6. The rdsRP according to claim 1, further comprising an alpha virus expression cassette.
7. The rdsRP according to claim 2, wherein the passenger gene encodes for an immunogen.
- 25 8. The rdsRP according to claim 1, wherein the dsRP is Phi-6, Phi-8, or Phi-13.
9. The rdsRP according to claim 2, wherein the rdsRP is expressed and amplified in a bacterial host strain.
- 30 10. The rdsRP according to claim 7, wherein the rdsRP further encodes for an adjuvant as an additional passenger gene.
11. The rdsRP according to claim 7, wherein the rdsRP further encodes for a cytokine.
- 35 12. The rdsRP according to claim 7, wherein the immunogen is foreign or endogenous.

13. The rdsRP according to claim 12, wherein the immunogen is foreign and is a member selected from the group consisting of viral proteins, bacterial proteins, parasite proteins, cytokines, chemokines, immunoregulatory agents, and therapeutic agents.

14. The rdsRP according to claim 13, wherein the immunogen originates from a viral pathogen, bacterial pathogen, or parasitic pathogens.

15. The rdsRP according to claim 14, wherein the immunogen originates from a viral pathogen comprising a member selected from the group consisting of Orthomyxoviruses, Retroviruses, Herpesviruses, Lentiviruses, Rhabdoviruses, Picornoviruses, Poxviruses, Rotavirus and Parvoviruses.

16. The rdsRP according to claim 13, wherein the viral protein is a member selected from the group consisting of: human immunodeficiency virus antigens, Nef, Rev, mutant derivatives of Tat, Tat-Δ31-45, Pol, T cell epitopes of gp120, and B cell epitopes of gp120, chimeric derivatives of HIV-1-CD4, chimeric Env-CD4, chimeric gp120-CD4, hepatitis B surface antigen, rotavirus antigens, influenza virus antigens, and herpes simplex virus antigens.

17. The rdsRP according to claim 12, wherein the immunogen is endogenous and is a member selected from the group consisting of cellular proteins, immunoregulatory agents, therapeutic agents, tumor immunogens, autoimmune immunogens and parts thereof.

18. The rdsRP according to claim 17, wherein the tumor immunogen comprises a member selected from the group consisting of PSA, CEA, MAGE-1 and tyrosinase.

19. The rdsRP according to claim 10, where the adjuvant comprises a member selected from the group consisting of: A subunit of cholera toxin, bacterial adenosine diphosphate-ribosylating exotoxins, pertussis toxin S1 subunit, adenylate cyclase-hemolysins of *Bordetella pertussis*, and parts thereof.

20. The rdsRP according to claim 11, wherein the cytokine comprises a member selected from the group consisting of; interleukin-4, IL-5, IL-6, IL-10, IL-12<sub>p40</sub>, IL-12<sub>p70</sub>, TGFβ and TNFα.

21. The rdsRP according to claim 2, wherein the IRES comprises a member selected from the group consisting of: the IRES located at nucleotides 665-1251 in pIRES2-EGFP, IRES from plasmid pCITE4a, IRES from plasmid pSVIRES-N, IRES of the 3'-untranslated region of the

mRNA for the beta subunit of mitochondrial H<sup>+</sup>-ATP synthase, (Accession #: Y11034), (Accession #: AF171227), (Accession #: Y07702), (Accession #: AJ000156) and (Accession #: D88622);

22. A composition comprising the rdsRP according to claim 4.

23. The composition according to claim 22, further comprising an alpha virus expression cassette.

24. The composition according to claim 22, wherein the passenger gene encodes for an immunogen.

25. The composition according to claim 22, wherein the dsRP is Phi-6, Phi-8, or Phi-13.

26. The composition according to claim 22, wherein the rdsRP is amplified in a bacterial host strain.

27. The composition according to claim 22, wherein the rdsRP further encodes for an adjuvant as a second passenger gene.

28. The composition according to claim 22, wherein the rdsRP further encodes for a cytokine.

29. The composition according to claim 22, wherein the immunogen is foreign or endogenous.

30. The composition according to claim 29, wherein the immunogen is foreign and is a member selected from the group consisting of viral proteins, bacterial proteins, parasite proteins, cytokines, chemokines, immunoregulatory agents, and therapeutic agents.

31. The composition according to claim 29, wherein the immunogen originates from a viral pathogen, bacterial pathogen, or parasitic pathogens.

32. The composition according to claim 31, wherein the immunogen originates from a viral pathogen comprising a member selected from the group consisting of Orthomyxoviruses, Retroviruses, Herpesviruses, Lentiviruses, Rhabdoviruses, Picornoviruses, Poxviruses, Rotavirus and Parvoviruses.

33. The composition according to claim 30, wherein the viral protein is a member selected from the group consisting of: human immunodeficiency virus antigens, Nef, Rev, mutant derivatives

of Tat, Tat-Δ31-45, Pol, T cell epitopes of gp120, and B cell epitopes of gp120, chimeric derivatives of HIV-1-CD4, chimeric Env-CD4, chimeric gp120-CD4, hepatitis B surface antigen, rotavirus antigens, influenza virus antigens, and herpes simplex virus antigens.

- 5     34.     The composition according to claim 29, wherein the immunogen is endogenous and is a member selected from the group consisting of cellular proteins, immunoregulatory agents, therapeutic agents, tumor immunogens, autoimmune immunogens and parts thereof.
- 10     35.     The composition according to claim 34, wherein the tumor immunogen comprises a member selected from the group consisting of PSA, CEA, MAGE-1 and tyrosinase.
- 15     36.     The composition according to claim 27, where the adjuvant comprises a member selected from the group consisting of: A subunit of cholera toxin, bacterial adenosine diphosphate-ribosylating exotoxins, pertussis toxin S1 subunit, adenylate cyclase-hemolysins of *Bordetella pertussis*, and parts thereof.
- 20     37.     The composition according to claim 28, wherein the cytokine comprises a member selected from the group consisting of; interleukin-4, IL-5, IL-6, IL-10, IL-12<sub>p40</sub>, IL-12<sub>p70</sub>, TGFβ and TNFα.
- 25     38.     A host cell transfected with the rdsRP of claim 4, wherein the rdsRP produces mRNA in the host cell that is recognized by the eukaryotic translation apparatus and expresses the passenger gene.
- 30     39.     The host cell according to claim 38, wherein the host cell comprises an eukaryotic or prokaryotic cell
40.     A method of vaccination, comprising administering to a subject the rdsRP according to claim 4 in an amount to express an effective amount of an encoded passenger gene.
- 35     41.     The method according to claim 40, wherein the dsRP is administered by intravenous, intramuscular, intradermal, intraperitoneally, intranasal or oral inoculation.
42.     The method according to claim 40, wherein the dsRP is administered with a non-pathogenic or attenuated bacterial vaccine vector.

43. A eukaryotic translation expression cassette comprising at least one segment of a double stranded RNA phage incorporating a nucleotide sequence encoding an IRES that is functionally linked to at least one gene of interest.
- 5 44. The eukaryotic translation expression cassette according to claim 43, wherein the segment of dsRP include segment L, segment M or segment S.
45. The eukaryotic translation expression cassette according to claim 43, further comprising an alpha virus expression cassette.
- 10 46. The eukaryotic translation expression cassette according to claim 43, wherein the passenger gene encodes for an immunogen.
47. The eukaryotic translation expression cassette according to claim 43, wherein the dsRP is
- 15 Phi-6, Phi-8, or Phi-13.
48. The eukaryotic translation expression cassette according to claim 43, wherein the eukaryotic translation expression cassette is introduced and amplified in a bacterial host strain.
- 20 49. The eukaryotic translation expression cassette according to claim 43, wherein the eukaryotic translation expression cassette further encodes for an adjuvant as a second passenger gene.
50. The eukaryotic translation expression cassette according to claim 43, wherein the
- 25 passenger gene encodes for a cytokine.
51. The eukaryotic translation expression cassette according to claim 46, wherein the immunogen is foreign or endogenous.
- 30 52. The eukaryotic translation expression cassette according to claim 51, wherein the immunogen is foreign and is a member selected from the group consisting of viral proteins, bacterial proteins, parasite proteins, cytokines, chemokines, immunoregulatory agents, and therapeutic agents.
- 35 53. The eukaryotic translation expression cassette according to claim 52, wherein the immunogen originates from a viral pathogen, bacterial pathogen, or parasitic pathogens.

54. The eukaryotic translation expression cassette according to claim 53, wherein the immunogen originates from a viral pathogen comprising a member selected from the group consisting of Orthomyxoviruses, Retroviruses, Herpesviruses, Lentiviruses, Rhabdoviruses, Picornoviruses, Poxviruses, Rotavirus and Parvoviruses.

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55. The eukaryotic translation expression cassette according to claim 52, wherein the viral protein is a member selected from the group consisting of: human immunodeficiency virus antigens, Nef, Rev, mutant derivatives of Tat, Tat-Δ31-45, Pol, T cell epitopes of gp120, and B cell epitopes of gp120, chimeric derivatives of HIV-1-CD4, chimeric Env-CD4, chimeric gp120-CD4, hepatitis B surface antigen, rotavirus antigens, influenza virus antigens, and herpes simplex virus antigens.

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56. The eukaryotic translation expression cassette according to claim 51, wherein the immunogen is endogenous and is a member selected from the group consisting of cellular proteins, immunoregulatory agents, therapeutic agents, tumor immunogens, autoimmune immunogens and parts thereof.

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57. The eukaryotic translation expression cassette according to claim 56, wherein the tumor immunogen comprises a member selected from the group consisting of PSA, CEA, MAGE-1 and tyrosinase.

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58. The eukaryotic translation expression cassette according to claim 49, where the adjuvant comprises a member selected from the group consisting of: A subunit of cholera toxin, bacterial adenosine diphosphate-ribosylating exotoxins, pertussis toxin S1 subunit, adenylate cyclase-hemolysins of *Bordetella pertussis*, and parts thereof.

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59. The eukaryotic translation expression cassette according to claim 52, wherein the cytokine comprises a member selected from the group consisting of; interleukin-4, IL-5, IL-6, IL-10, IL-12<sub>p40</sub>, IL-12<sub>p70</sub>, TGFβ and TNFα.

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60. The eukaryotic translation expression cassette according to claim 43, wherein the IRES comprises a member selected from the group consisting of: the IRES located at nucleotides 665-1251 in pIRES2-EGFP, IRES from plasmid pCITE4a, IRES from plasmid pSVIRES-N, IRES of the 3'-untranslated region of the mRNA for the beta subunit of mitochondrial H<sup>+</sup>-ATP synthase, (Accession #: Y11034), (Accession #: AF171227), (Accession #: Y07702), (Accession #: AJ000156) and (Accession #: D88622);

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61. The eukaryotic translation expression cassette according to claim 43, further comprising a passenger gene encoding for green fluorescent protein.

62. A method of inducing an immune response or biological activity comprising administering to a subject the rdsRP according to claim 4 in a sufficient amount to express an effective amount of encoded passenger gene.

63. The method according to claim 62, wherein the rdsRP is delivered to mammalian cells or tissues via a bacterial vector.

64. The method according to claim 62, wherein the segment of dsRP include segment L, segment M or segment S.

65. The method according to claim 62, wherein the dsRP further comprises an alpha virus expression cassette.

66. The method according to claim 62, wherein the passenger gene encodes for an immunogen.

67. The method according to claim 62, wherein the dsRP is Phi-6, Phi-8, or Phi-13.

68. The method according to claim 62, wherein the rdsRP further encodes for an adjuvant as an additional passenger gene.

69. The method according to claim 66, wherein the immunogen is foreign or endogenous.

70. The method according to claim 69, wherein the immunogen is foreign and is a member selected from the group consisting of viral proteins, bacterial proteins, parasite proteins, cytokines, chemokines, immunoregulatory agents, and therapeutic agents.

71. The method according to claim 69, wherein the immunogen originates from a viral pathogen, bacterial pathogen, or parasitic pathogens.

72. The method according to claim 71, wherein the immunogen originates from a viral pathogen comprising a member selected from the group consisting of Orthomyxoviruses, Retroviruses, Herpesviruses, Lentiviruses, Rhabdoviruses, Picornoviruses, Poxviruses, Rotavirus and Parvoviruses.

73. The method according to claim 70, wherein the viral protein is a member selected from the group consisting of: human immunodeficiency virus antigens, Nef, Rev, mutant derivatives of Tat, Tat-Δ31-45, Pol, T cell epitopes of gp120, and B cell epitopes of gp120, chimeric derivatives of HIV-1-CD4, chimeric Env-CD4, chimeric gp120-CD4, hepatitis B surface antigen, rotavirus antigens, influenza virus antigens, and herpes simplex virus antigens.

74. The method according to claim 69, wherein the immunogen is endogenous and is a member selected from the group consisting of cellular proteins, immunoregulatory agents, therapeutic agents, tumor immunogens, autoimmune immunogens and parts thereof.

75. The method according to claim 74, wherein the tumor immunogen comprises a member selected from the group consisting of PSA, CEA, MAGE-1 and tyrosinase.

76. The method according to claim 68, where the adjuvant comprises a member selected from the group consisting of: A subunit of cholera toxin, bacterial adenosine diphosphate-ribosylating exotoxins, pertussis toxin S1 subunit, adenylate cyclase-hemolysins of *Bordetella pertussis*, and parts thereof.

77. The method according to claim 70, wherein the cytokine comprises a member selected from the group consisting of; interleukin-4, IL-5, IL-6, IL-10, IL-12<sub>p40</sub>, IL-12<sub>p70</sub>, TGFβ and TNFα.

78. The method according to claim 62, wherein the IRES comprises a member selected from the group consisting of: the IRES located at nucleotides 665-1251 in pIRES2-EGFP, IRES from plasmid pCITE4a, IRES from plasmid pSVIRES-N, IRES of the 3'-untranslated region of the mRNA for the beta subunit of mitochondrial H<sup>+</sup>-ATP synthase, (Accession #: Y11034), (Accession #: AF171227), (Accession #: Y07702), (Accession #: AJ000156) and (Accession #: D88622);

79. A live bacteria comprising at least one dsRP according to claim 4.

80. A method of generating the dsRP expression cassette according to claim 1, comprising: incorporating an IRES that is functionally linked to a downstream immunogen into at least one segment of a dsRP.